

Article

Dissolved Organic Matter as Affected by Forms and Doses of Nitrogen Applied to Soils of Temperate Deciduous Forests in China

Peng Wang ^{1,†}, Minghua Song ^{2,†} and Chunmei Wang ^{1,*} ¹ College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China² Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, A11, Datun Road, Chaoyang District, Beijing 100101, China

* Correspondence: wangcm@bjfu.edu.cn; Tel.: +86-10-6233-6615; Fax: +86-10-6233-6596

† These authors contributed equally to this work.

Abstract: Dissolved organic matter (DOM) is an important component in the biogeochemical cycles of elements like nitrogen (N) and carbon. The aim of this study was to elucidate the effect of long-term inorganic N addition on the quantity and quality of DOM in forest soils. A field study was conducted on three forms of inorganic N, namely (NH₄)₂SO₄, NH₄NO₃, and NaNO₃, applied at low (50 kg N ha⁻¹) or high (150 kg N ha⁻¹) annual doses from 2011 to 2019. The total dose was split into eight equal monthly doses applied during the growing season (from March to October). Both the form and the dose increased the content of dissolved organic carbon (DOC) in soil, the strongest effect being that of NaNO₃. However, the higher dose had a weaker effect because of N enrichment. UV-visible (UV-vis) and excitation-emission matrix (EEM) spectroscopy showed that the addition of N made DOM more aromatic and increased the degree of humification. EEM-parallel factor analysis (PARAFAC) modelling suggested that DOM in the forest soils mainly contained a fulvic-like constituent (C1), humic-like substances (C2), and aromatic protein-like components (C3). The addition did not change the position of the DOM fluorophore in the soil but affected the proportions of the three PARAFAC-derived components (increasing those of C1 and C2 but decreasing that of C3), indicating that long-term addition of N may amplify the decrease in protein-like constituents of surface soil. Hence, N addition increased the complexity of the DOM structure.

Keywords: nitrogen deposition; temperate forest soil; dissolved organic matter; UV-vis spectroscopy; EEM-PARAFAC



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1. Introduction

Dissolved organic matter (DOM) comprises organic molecules with different dimensions and structures, operationally considered as the fraction that can pass through a membrane with a pore size of 0.45 μm [1]. Although DOM accounts for less than 5% of the soil organic matter by weight [2], it is the most movable and reactive fraction of carbon (C) in forest soils [3], and is a critical component of biogeochemical cycles in regulating soil respiration, plant nutrient absorption and microbial metabolism of receiving aquatic systems. Among the components of DOM, protein-like components are considered more biologically active than the humic- and fulvic-like components [4,5]. As the essential source of energy for forest ecosystems [6], DOM plays a significant role in the C and nutrient budgets of forest soils [7].

Deposition of nitrogen (N) is one of the main outcomes of global climate change [3]. Over the past 150 years, global annual anthropogenic emissions of reactive N have increased from 15 to 187 Tg [8] and are likely to double by 2050 [9]. The annual average rate of N deposition in China increased to 21.1 kg·ha⁻¹ in the 2000s, making the country the

third largest in the world in terms of N deposition [10]. The deposition has changed the biogeochemical cycles of terrestrial ecosystems and has affected plant productivity [11], structure of microbial communities [12,13], and soil respiration [8]. More important, long-term N deposition has also greatly altered the composition and structure of DOM in soils [2], inevitably changed many important biogeochemical processes in forest soil, and changed the quality and quantity of DOC input and metabolism in the downstream of aquatic ecosystem.

Nitrogen is a key component for microbial activity [14], and most DOM is of microbial origin [15]. Consequently, the liberation of DOC is believed to be affected by changes in the availability of N [16]. A global study indicated that the concentration of DOC was related to the C/N ratio and NH_4^+ -N levels in soils [17]. For example, Hyvönen et al. [18] reported that the addition of N increased the amount of soil organic carbon, resulting in higher contents of DOC in forest soils. Yuan et al. [2], on the other hand, suggested that the addition of N inhibits soil respiration and thus lowers the content of DOC. Yet other researchers maintain that the release of DOC in forest soils is connected neither to N conditions [19] nor to the application of N fertilisers [20,21]. Thus, the effect of N addition on the contents of DOM is still controversial.

In forest ecosystems, the addition of N may change the structure and the constituents of DOM by influencing the process of humification [16]. As mentioned above, experiments on the topic have given conflicting results. For example, Yuan et al. [2] suggested that addition of N enhances the degree of aromatic and macromolecular compounds, making their structure more complicated, whereas Xu et al. [22] reported that application of N fertilisers makes the structure of DOM simpler. It should be noted that the above studies were mostly concentrated in subtropical areas with different soil types but with only one form of N. To better understand and predict the effects of the deposition of atmospheric N on DOM in soils of temperate forests, we need long-term field experiments involving different doses and multiple forms of N.

Ultraviolet-visible (UV-vis) spectroscopy and excitation-emission matrices (EEM) spectroscopy are commonly used for identifying and quantifying the components of DOM [23], and combining parallel factor (PARAFAC) modelling with EEM spectroscopy can reduce the influence of overlapping peaks in the initial fluorescence spectra and thus reveal the composition of DOM in greater detail [24,25]. However, till date, few studies have explored the effect of adding N on DOM in forest soils using EEM-PARAFAC modelling—or, for that matter, using any other method [26]. It was to contribute to filling this gap in knowledge that we planned an eight-year field experiment comprising two doses and three forms of N added to the soil from a temperate forest to examine the effect of N on the quantity and quality of DOM by determining its chemical and structural properties using UV-vis and EEM-PARAFAC. We hypothesized that (1) N inputs to forest ecosystems may increase DOM content by increasing the decomposition of SOM and that this promotion effect likely increased with the N addition level; (2) long-term N addition may increase the complexity of the DOM structure and the degree of humification. The aims of the study were to provide insight into the characteristics of DOM responding to the case of applied N with different chemical forms and doses, which might give a better understanding of the effect of atmospheric N deposition on the C cycle in forest ecosystems.

2. Materials and Methods

2.1. Site and Experimental Design

The study was carried out in a temperate forest of the Xi Mountain Research Station (Figure 1a) of Beijing Forestry University ($31^\circ 54' 32''$ N, $110^\circ 68' 08''$ E; elevation, 133 m), China. The site has a temperate semi-humid continental monsoon climate. July is the hottest and the rainiest month. The annual average temperature and rainfall over the past 20 years were 11.6°C and 638.3 mm (Figure 1b) [27]. The predominant species is *Quercus liaotungensis*, with the following typical values: diameter at breast height, 9.7 cm; canopy density, 69%; average height, 8.4 m; and plant density, 2963 trees ha^{-1} [8]. According to the

World Reference Base for Soil Resources soil classification, the soil in the study area is a Chromic Luvisol, consisting of 51% sand, 40% silt, and 9% clay [28]. More details of soil properties are given in Table 1. The experimental plots, laid out at random, did not differ significantly in their soil properties before the addition of N.

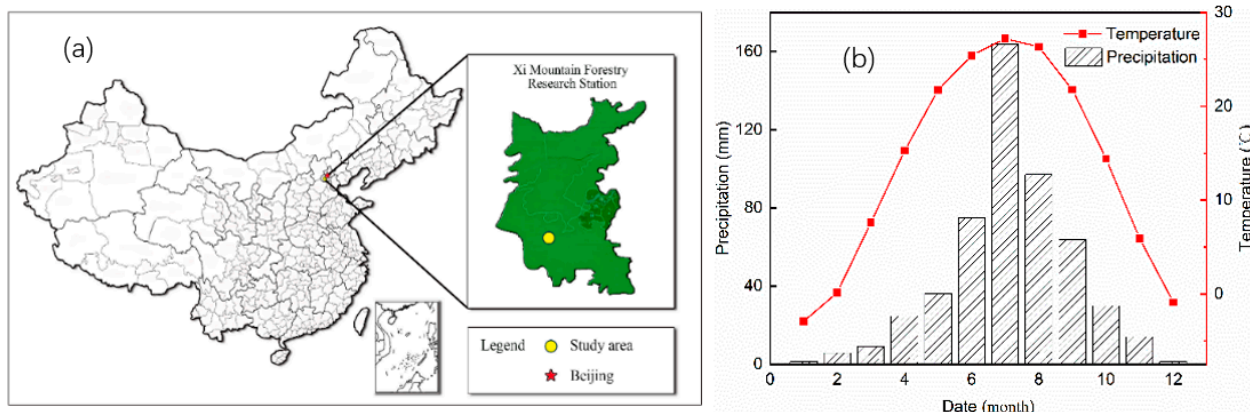


Figure 1. Geographical location (a) and the temperature and precipitation (b) of the study site.

Table 1. Physical-chemical properties in the upper 15 cm of soil before N addition ($n = 3$).

Soil Property	Bulk Density (g cm^{-3})	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	C/N
Value	1.21 ± 0.32	7.13 ± 0.21	29.78 ± 1.06	2.42 ± 0.88	2.48 ± 0.47	12.95 ± 1.28	12.25 ± 0.42

In March 2011, seven plots, each measuring 10×10 m and dominated by *Q. liaotungensis*, were chosen for the experiment. Each set of the seven plots formed one block, and the entire experiment comprised three such blocks, each corresponding to one replication. Within each block, the seven treatments were assigned at random. A 1.5 m wide buffer zone separated each plot from the next to avoid any interference from the adjacent treatment. The seven treatments comprised a control with no addition of N and two doses of N, low or high (50 kg or 150 kg of N given annually per hectare), given either as $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , or NaNO_3 . The two doses were meant to simulate approximately two times and five times the actual annual deposition of N in the study region ($30.6 \text{ kg N ha}^{-1}$). The actual bulk deposition of NH_4^+ -N was, on average, 2.1 times the NO_3^- -N deposition through atmospheric deposition in the study area. Based on local actual N forms of atmosphere deposition and experiments conducted by peer researchers, the above three N forms were used [8]. Each year from March 2011 to July 2019, the annual dose of N was split into eight equal doses applied monthly during the growing period (from March to October) 1 day after sampling the soil. The required quantity of N was dissolved in distilled water and the solution was sprayed on the soil surface. For the control, only distilled water was sprayed, the total volume of water being the same for the control and for all the treatments.

2.2. Sampling and Analysis

Soil samples, 0–15 cm in depth, were collected from 12 random points using 3.5 cm diameter cores in July 2019, 8 years after initiating the experiment [8]. The soil cores were brought back to the laboratory immediately, stored at 4°C , and the soil was analysed within 48 h. The air-dried soil samples were mixed, ground, and passed through a 2 mm sieve for removing roots and other residues. Dissolved organic matter from the samples was extracted from the fresh soil samples (equivalent to 5 g oven-dried) with 1:5 soil/water ratio by shaking the soil suspension for 24 h. After centrifugation at 4000 rpm for 20 min, the supernatants were filtered through a $0.45 \mu\text{m}$ membrane (Whatman, Clifton, NJ, USA). The

filtrate was stored in glass bottles at 4 °C, and DOC was measured using a TOC analyser (multi N/C 3100, Analytik Jena, Jena, Germany).

2.3. Characterizations

2.3.1. UV-Visible Spectroscopy

The filtrate was subjected to UV-visible (UV-vis) spectroscopy (240–400 nm) using a spectrophotometer (U-3900, Hitachi, Ibarakiken, Japan) and quartz cells with a 1 cm path length and Milli-Q water as a blank. To reduce the effect of internal filters, samples with high UV-vis absorbance values (as high as 1.50–3.00) were diluted with Milli-Q water to the point at which the absorbance at 300 nm was 0.02 [29,30]. To acquire more details of the DOM from forest soils from the spectra, three indicators were calculated, namely spectral slope coefficient ($S_{275-295}$, μm^{-1}), UV absorbance at 254 nm ($SUVA_{254}$, $\text{L mg C}^{-1} \text{m}^{-1}$), and the ratio of UV absorbance at 253 nm to that at 203 nm (A_{253}/A_{203}).

It is known that $S_{275-295}$ is negatively correlated with the molecular weight of organic material [31] and can be computed from the non-linear least-square regression of the absorption coefficients $\alpha(\lambda)$ and the wavelength (from 275 nm to 295 nm) (Equations (1) and (2)) [29]:

$$\alpha_{\lambda} = 2.303A_{\lambda}/l \quad (1)$$

$$\alpha_{\lambda} = \alpha_{\lambda_0} e^{S(\lambda_0 - \lambda)} + K \quad (2)$$

where λ (nm) is the chosen wavelength (from 275 nm to 295 nm); α_{λ} (m^{-1}) is the absorption coefficient; A_{λ} is the absorbance; l is the optical path length (0.01 m); λ_0 is the reference wavelength (300 nm); S is the spectral slope coefficient (μm^{-1}); and K is a background parameter.

The second indicator, $SUVA_{254}$, is widely used for assessing the aromaticity of DOM: the higher values of $SUVA_{254}$ indicate that aromatic substances are present in larger numbers in the soil [25]. The value of $SUVA_{254}$ was calculated by dividing the UV absorbance at 254 nm by the content of DOC (Equation (3)) [32]:

$$SUVA_{254} = (A_{254}/C_{\text{DOC}}) \times 100 \quad (3)$$

where A_{254} is the absorbance at the wavelength of 254 nm and C_{DOC} is the content of DOC ($\text{mg}\cdot\text{L}^{-1}$).

The third indicator, A_{253}/A_{203} , reflected the extent to which aromatic rings in organic compounds had been replaced [33] and was calculated by dividing UV absorbance at 253 nm by that at 203 nm.

2.3.2. Excitation-Emission Matrix Fluorescence Spectroscopy

The fluorescence characteristics of DOM were determined using a spectrophotometer (F-7000, Hitachi, Ibarakiken, Japan). The excitation and emission slits were both set as 5 nm and the scanning range for both excitation and emission were 200–550 nm. Fluorescence intensities were collected at intervals of 5 nm along the excitation and emission wavelengths at a scanning speed of 1200 nm min^{-1} . Milli-Q water was applied as a blank to remove the water Raman scatter peaks [29].

The humification index (HIX) is a ratio of two specific spectral region areas (H and L) of the emission spectrum scanned at an excitation wavelength of 254 nm (Equation (4)) [34]:

$$\text{HIX} = H/L \quad (4)$$

where H is the spectral region with emission wavelengths between 300 nm and 345 nm and L is the spectral region with emission wavelengths between 435 nm and 480 nm.

2.3.3. PARAFAC Modelling

A PARAFAC model can methodically decompose and extract individual components of DOM from any EEM fluorescent data set and shows the relative contents of different organic components in every sample [25]. All the EEM spectral data are composed of a three-way array of X with $S \times I \times J$, where S , I , and J define the number of samples, emission wavelength, and excitation wavelength, respectively. The model (216 samples \times 55 emissions \times 55 excitations) can be described using Equation (5):

$$x_{sij} = \sum_{f=1}^F a_{sf} b_{if} c_{jf} + e_{sij} \text{ for } s = 1 \dots S, i = 1 \dots I, j = 1 \dots J \quad (5)$$

where F represents the number of fluorophores; x_{sij} is the fluorescent peak of the s_{th} sample at specific emission i or excitation j wavelength; a_{sf} is proportional to the concentration of the f_{th} organic component in the s_{th} sample; b_{if} and c_{jf} are judgements of the emission and excitation spectrum, respectively, for the f_{th} organic component; and e_{sij} is the residual matrix applied to minimise the sum of squared residuals by transforming the least-squares algorithm [35].

2.4. Statistical Analyses

Data analysis was conducted mainly using Origin 2018 and SPSS ver. 26.0. Before one-way analysis of variance (ANOVA), data were checked for normality using the Levene and Mauchly's test. One-way ANOVA and the least significant difference test were applied to confirm the influences of the form and the dose of N and their possible interplay on the concentration of DOC. EEM-PARAFAC analysis was accomplished applying the DOMFluor toolbox in MATLAB R2010a. Split-half analysis was applied to confirm the accuracy of the decomposed data from PARAFAC modelling, and data on the constituents of DOM were exported using MATLAB.

3. Results and Discussion

3.1. Effect of N Addition on Soil Available Nitrogen and Dissolved Organic Carbon

Soil available N was significantly affected by N form, N level, and their interaction (Table 2). The overall effect of N addition on soil NH_4^+ -N and NO_3^- -N was positive, with an average increase of 41.89% and 110.18%, respectively. Soil NH_4^+ -N and NO_3^- -N content reached the maximum value of 4.59 and 26.47 $\text{mg}\cdot\text{kg}^{-1}$ under $(\text{NH}_4)_2\text{SO}_4$ and NaNO_3 treatment, respectively, in 2019 (Table 2). Both low- and high-level N addition significantly stimulated available N content, and high level of N addition exerted significantly stronger effects on NO_3^- -N content than that of low level (Table 2).

Table 2. Average NH_4^+ -N and NO_3^- -N content in control and N addition plots.

	Average NH_4^+ -N Content ($\text{mg}\cdot\text{kg}^{-1}$)	Increase from Control		Average NO_3^- -N Content ($\text{mg}\cdot\text{kg}^{-1}$)	Increase from Control
Control	2.97 \pm 0.28 b	—	Control	11.72 \pm 1.29 c	—
Low	4.31 \pm 0.31 a	45.12%	Low	21.55 \pm 1.54 b	83.87%
High	4.24 \pm 0.35 a	42.76%	High	28.72 \pm 3.15 a	145.05%
Control	2.97 \pm 0.28 c		Control	11.72 \pm 1.29 c	
NaNO_3	3.78 \pm 0.02 b	27.27%	NaNO_3	26.47 \pm 5.94 a	125.85%
$(\text{NH}_4)_2\text{SO}_4$	4.59 \pm 0.08 a	54.55%	$(\text{NH}_4)_2\text{SO}_4$	21.29 \pm 3.40 b	81.66%
NH_4NO_3	4.15 \pm 0.11 ab	39.73%	NH_4NO_3	25.13 \pm 3.58 a	114.47%

Note: Different lowercase letters represent significant differences ($p = 0.05$).

Nitrogen in all the three forms and both the doses raised the content of DOC in the samples substantially (Figure 2), from 110.00 $\text{mg}\cdot\text{kg}^{-1}$ in the control to 129.25–235.07 $\text{mg}\cdot\text{kg}^{-1}$ in the treatments. These results were consistent with those from many earlier studies [36–39]. In the present study, the amounts of above-ground and below-ground biomass C following the addition of N increased significantly, by 29.00% and 16.11% on average, respectively, compared

to the control (unpublished data). Tipping et al. [40] suggested that higher productivity of forests supplied with N increases the amount of DOC stored, its source being the diverse organic materials. The addition of N also alters the production of microbial DOM by promoting or inhibiting the activities of specific microorganisms [41]. Wang et al. [6] showed that the addition of N led to a moderate degradation of organic matter and the accumulation of tissue softened by rotting, the contents of which are more easily soluble in water. In the present study, adding N lowered the soil pH from 7.13 to 6.71–5.44. Macalady et al. [21] suggested that adding N changes the solute chemistry in many ways, including increasing ion strength and acidity, thereby reducing the amount of DOC lost through leaching; the same result is achieved also by the more acidic conditions [21,42], thereby leading to accumulation of DOC in larger quantities [6].

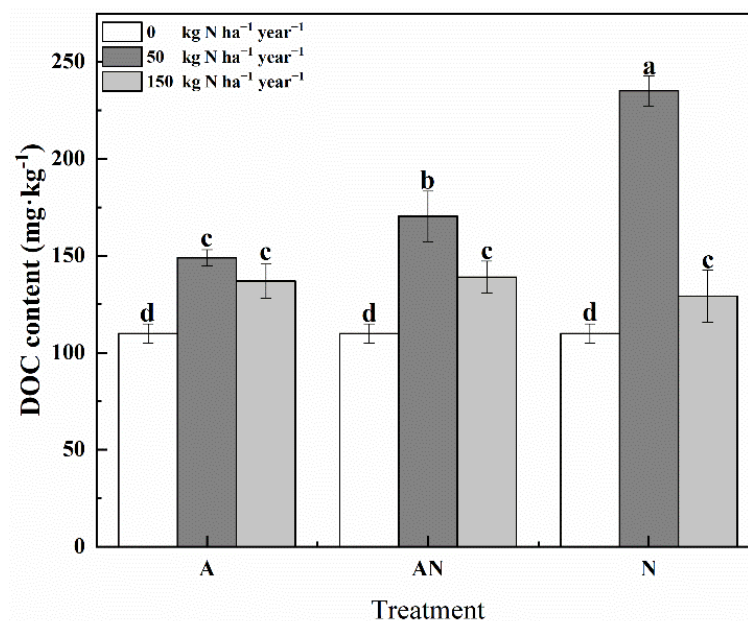


Figure 2. Content of dissolved organic carbon under different N addition. Vertical bars represent \pm SE ($n = 3$). A, $(\text{NH}_4)_2\text{SO}_4$; AN, NH_4NO_3 ; N, NaNO_3 . Means with different lowercase letters are significantly different at $p < 0.05$.

Compared to the control, the lower dose of N increased DOC content by approximately 55% on average, whereas the corresponding figure for the higher dose was a little above 13% (Table 3). This difference was probably due to N enrichment at different stages. In the study region, N was the limiting factor in the initial years and its supply increased the tree biomass [8,43]. The effects of N addition on the priming effect could be well explained by the “N mining” mechanism: The removal of energy limitation of microorganisms after fresh litter input enabled them to produce energetically expensive and N demanding extracellular enzymes to decompose soil organic matter for nutrient acquisition (such as N) [44]. However, as the experiment continued, the priming effect declined gradually when the soil was relieved from N limitation. Fresh litter and N addition supplied not only C and energy but also other nutrients (such as N) for microorganisms, the amounts of which could meet microbial demands and impeded their need to mine N from soil organic matter. Therefore, “stoichiometric theory” might be applicable when the litter input contained not only C but also N [45]. The accumulation of N weakened the N-induced priming effect [46], which meant that smaller quantities of DOC were released, leading to the accumulation of refractory substances [47]. We believe that the accumulation of N in ever increasing amounts will lower the content of DOC in soil in the future.

Table 3. Average DOC content in control and N addition plots.

	Average DOC Content (mg·kg ⁻¹)	Increase from Control		Average DOC Content (mg·kg ⁻¹)	Increase from Control
Control	110.00 ± 4.92 a	—	Control	110.00 ± 4.92 a	—
Low	184.82 ± 11.09 c	54.99%	(NH ₄) ₂ SO ₄	133.00 ± 9.30 b	40.62%
High	135.07 ± 8.78 b	13.27%	NH ₄ NO ₃	154.68 ± 11.60 c	20.91%
			NaNO ₃	182.16 ± 9.11 d	65.60%

(NH₄)₂SO₄ = average DOC content in both low and high (NH₄)₂SO₄ addition plots (NH₄NO₃ and NaNO₃ were calculated in the same way). Low = average DOC content in L-(NH₄)₂SO₄, L-NH₄NO₃ and L-NaNO₃ plots (high was calculated in the same way). Means with different lowercase letters in the same column are significantly different at $p < 0.05$.

The content of DOC varied with the form of N, the average of the two doses being 133.00 ± 9.30 mg·kg⁻¹ for (NH₄)₂SO₄, 154.68 ± 11.60 mg·kg⁻¹ for NH₄NO₃, and 182.16 ± 9.11 mg·kg⁻¹ for NaNO₃, with the corresponding increase in DOC being approximately 21%, 40%, and 66% (Table 3). When the dose was low (50 kg N ha⁻¹), the form of N exerted a significant effect on soil DOC content (Table 4). A recent global meta-analysis indicated that nitrification is suppressed as the ratio of NH₄⁺ to NO₃⁻ increases following the deposition of N deposition, with NH₄⁺ being the dominant form. More important, the content of DOC was found to be strongly controlled by the form of N, with NO₃⁻-N exerting a greater effect than NH₄⁺-N [39,48], a finding consistent with our result.

Table 4. Results of one-way ANOVA and LSD test on the effects of time, N form and level, and their interactions on cumulative DOC content.

Subjects	d.f.	F	p
N form	2	10.267	0.002
N level	1	110.856	0.000
N form × N level	2	42.855	0.000

3.2. Characterization of Parameters Using UV-Visible Spectroscopy and Excitation-Emission Matrix Fluorescence Spectroscopy

No prominent absorption peaks were found in the UV-vis spectra, although the different treatments resulted in a marked red-shift (Figure 3), which shows that the addition of N can increase the soil DOM chromophore, thereby increasing its light absorption capacity, and that the conjugate structure of soil DOM and the degree of humification are also increased [49]. As illustrated in Table 5, the value of $S_{275-295}$ for the control was 14.59 μm⁻¹ whereas that in the treatments was 11.60–13.95 μm⁻¹. The increase points to the greater molecular weight of the DOM, which may be because it contained more double bonds and became more aromatic [6]. Substituted aromatic ring structures generally result in higher values of A_{253}/A_{203} , which suggests that the organic components contain a greater number of polar functional groups (i.e., carboxylic, carbonyl, hydroxyl, and ester groups) on aromatic rings [50]. The values of A_{253}/A_{203} in the treatments ranged from 0.22 to 0.37; the higher values imply that the DOM in soils to which N had been added contained a greater number of substitute functional groups and aliphatic chain structures, probably because of the increased contents of reactive groups (carboxyl, hydroxyl, carbonyl, ester, etc.) in the aromatic ring substituents of the weakly acidic environment [50].

The increase in specific ultraviolet absorbance (SUVA) indicates the high concentration of aromatic compounds, because SUVA values are positively related to the aromatic carbon content of DOM [26]: $SUVA_{254}$ values of soil samples from the treatments varied from 2.57 to 9.76 (Table 4), similar to those reported by Yuan et al. [2]. Ogawa et al. [51] demonstrated that microbial processes change the molecular structure of DOM. The increase in SUVA may have been due to the preferential use of non-aromatic groups by microorganisms or their ability to transform labile compounds to aromatic carbon structures, or a

combination of both [26,52]; the increase may also have been due to the rapid transformation of perishable compounds by microorganisms, resulting in products containing more unsaturated carbon bonds that absorb UV light at 254 nm [53]. Qin et al. [54] reported significant negative correlations between $S_{275-295}$ and SUVA values, an observation that matches the results of our study. The increase in HIX (from 4.51 to 8.28) in the treatments may be because the microbial activity changed the fluorescent components associated with longer wavelengths and/or structural modification [55].

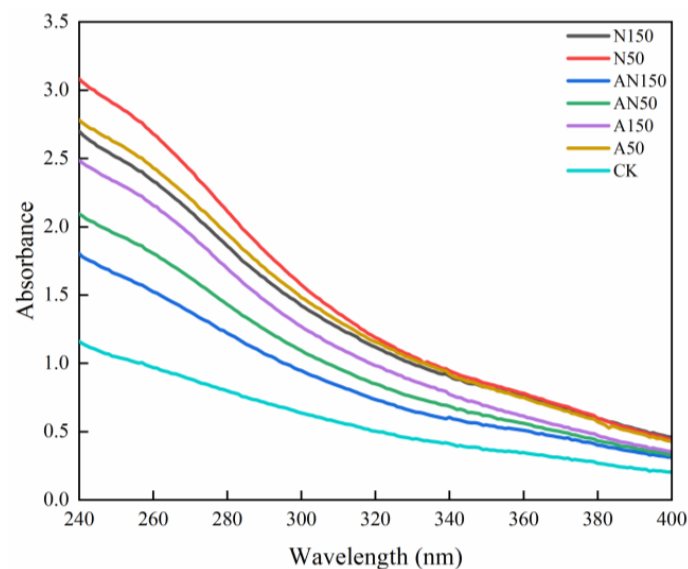


Figure 3. UV-vis spectra of DOM extracted from temperate forestry soil samples under different treatment. A, $(\text{NH}_4)_2\text{SO}_4$; AN, NH_4NO_3 ; N, NaNO_3 . 50, 50 kg N ha^{-1} year $^{-1}$; 150, 50 kg N ha^{-1} year $^{-1}$.

Table 5. The average values of characterization parameters calculated by UV-vis and EEM spectral data ($n = 21$).

Treatment	S (μm^{-1})	SUVA ₂₅₄ ($\text{L mg C}^{-1} \text{m}^{-1}$)	A_{253}/A_{203}	HIX
CK	14.59 (0.21)	2.57 (0.15)	0.22 (0.09)	4.51 (0.18)
A50	11.60 (0.16)	9.76 (0.21)	0.37 (0.11)	8.16 (0.23)
A150	12.81 (0.22)	6.10 (0.26)	0.32 (0.08)	5.16 (0.21)
AN50	13.33 (0.15)	4.98 (0.18)	0.33 (0.13)	6.69 (0.19)
AN150	13.95 (0.28)	9.18 (0.14)	0.34 (0.06)	6.17 (0.22)
N50	13.89 (0.17)	7.79 (0.16)	0.34 (0.12)	4.80 (0.17)
N150	13.32 (0.11)	6.90 (0.13)	0.30 (0.07)	8.28 (0.28)

A, $(\text{NH}_4)_2\text{SO}_4$; AN, NH_4NO_3 ; N, NaNO_3 . 50, 50 kg N ha^{-1} year $^{-1}$; 150, 150 kg N ha^{-1} year $^{-1}$. HIX, humification index. All values are reported as “mean (standard deviation)” based on measurement results for triplicated samples.

3.3. PARAFAC Analysis of Temperate Forest Soil DOM

The three-dimensional fluorescence (or EEM) spectra combined with PARAFAC modelling were applied to identify different components of DOM in surface soil from the temperate forest. The model identified three organic components that differed between the samples from the control (Figure 4) and those from the treatments (Figure 5). Split-half analysis showed the selection of the factor number to be reasonable (Figures 6 and 7). The three organic constituents found in samples from the control and treatments, referred to as model A and model B, respectively, comprised a fulvic-like constituent (C1), a humic-like constituent (C2), and a protein-like constituent (C3).

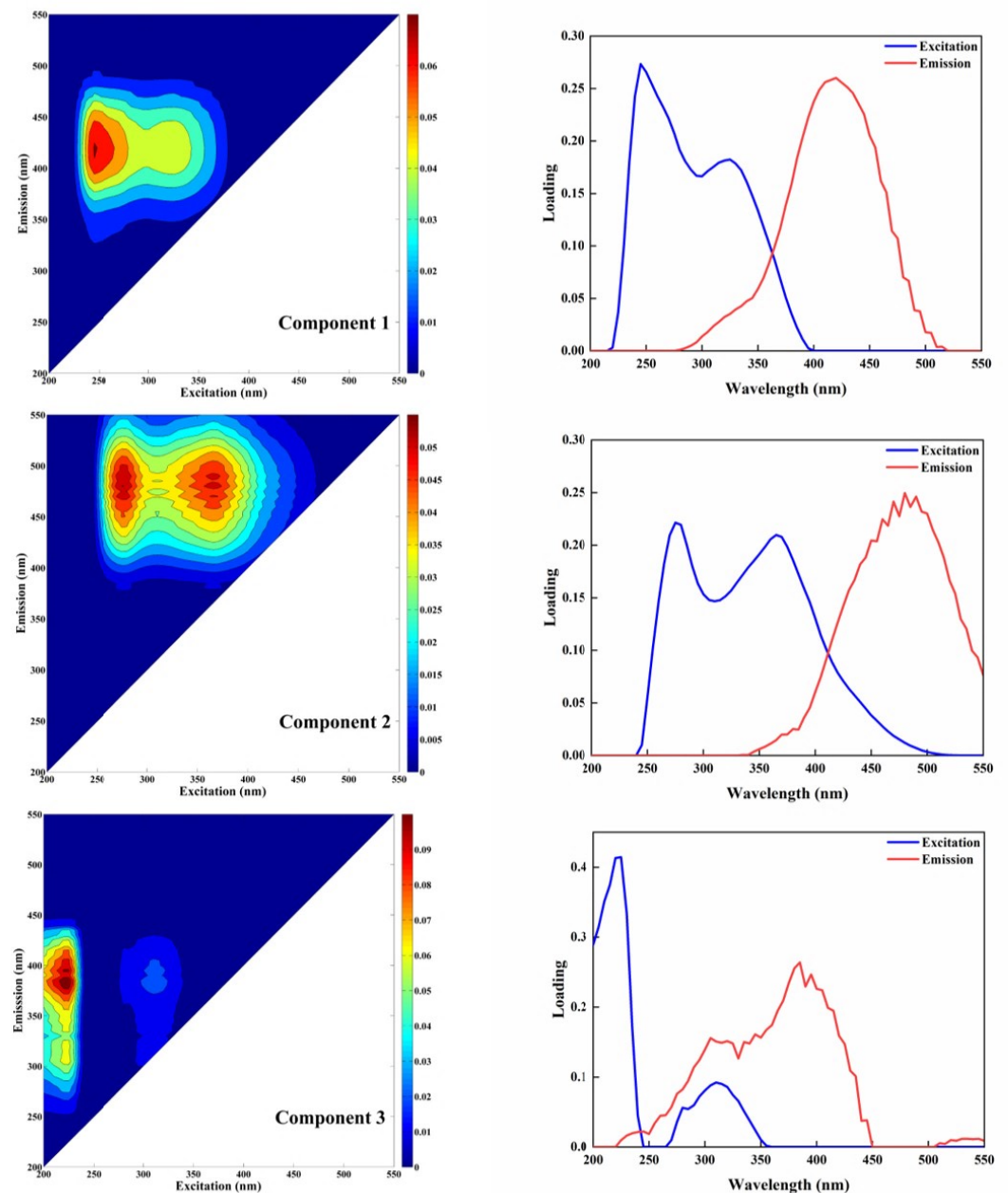


Figure 4. PARAFAC model A. Spectral characteristic EEM images of fluorescent components identified using EEM-PARAFAC analysis of temperate forest soil sample.

The fulvic-like constituent showed one peak at Ex/Em wavelengths of 245/420 nm, respectively. This spectral characteristic was identified as that of the fulvic-like fluorescence. The humic-like constituent showed a primary and a secondary peak, at 275/480 nm (Ex/Em) and 365/480 (Ex/Em), respectively, suggesting that the component was similar to humic acid fluorophores. The protein-like component was identified with two fluorescence peaks: the bottom peak (225/305 nm) stood for the tyrosine-like peak B components and the top peak (225/375 nm), for the tryptophan-like peak T components. Lee et al. [56] reported that organic components fluorescent at long wavelengths are related to high aromatic polycondensation structural organic matter, whereas fluorophores at short wavelengths indicate the presence of low aromatic organic components. Compared to C1 and C3, the peak position of C2 was shifted towards longer wavelengths. We speculate that the C2 organic components were related to the larger molecular weight and extremely condensed structures, indicating greater stability.

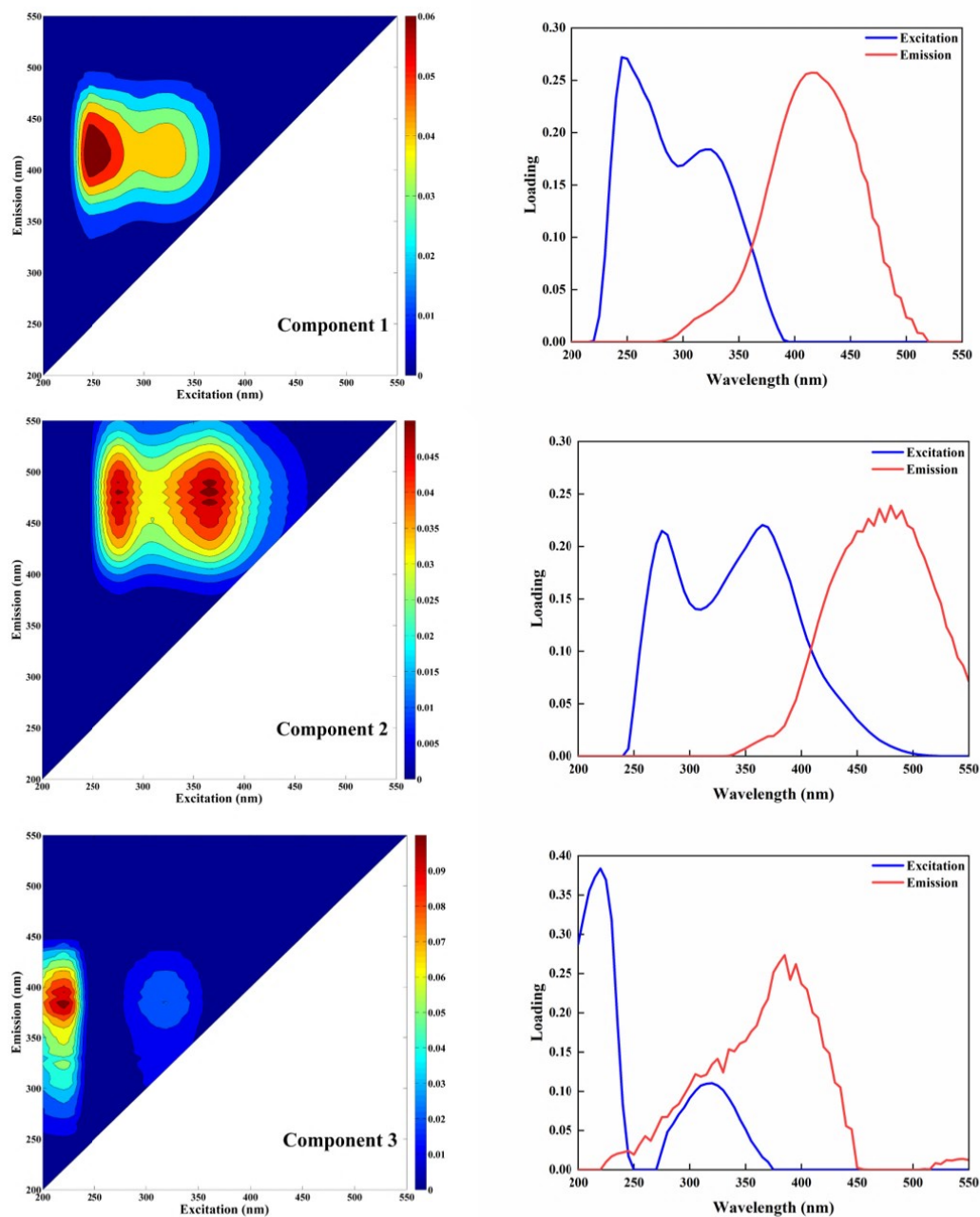


Figure 5. PARAFAC model B. Spectral characteristic EEM images of fluorescent components identified using EEM-PARAFAC analysis of temperate forest soil sample with the addition of nitrogen.

The addition of N did not affect the position of the DOM fluorescent group (Figure 6) but did change the proportions of the three PARAFAC-derived components (Figure 8). Specifically, the proportions of C1 and C2 tended to increase whereas those of C3 tended to decrease. These trends were probably the result of a greater number of polycondensation structures being formed by the utilization of unstable chemical compounds by microorganisms [52]. This change shows that the structure of DOM in soils to which N has been added tends to be more complicated and stable. In general, long-term addition of N probably increases the possibility of the loss protein-like constituents from surface soil and also increases the degree of soil humification. At the same time, the long-term accumulation of N might also lead to the massive accumulation of non-biodegradable substances, resulting in difficulty in the utilization of soil nutrients [2].

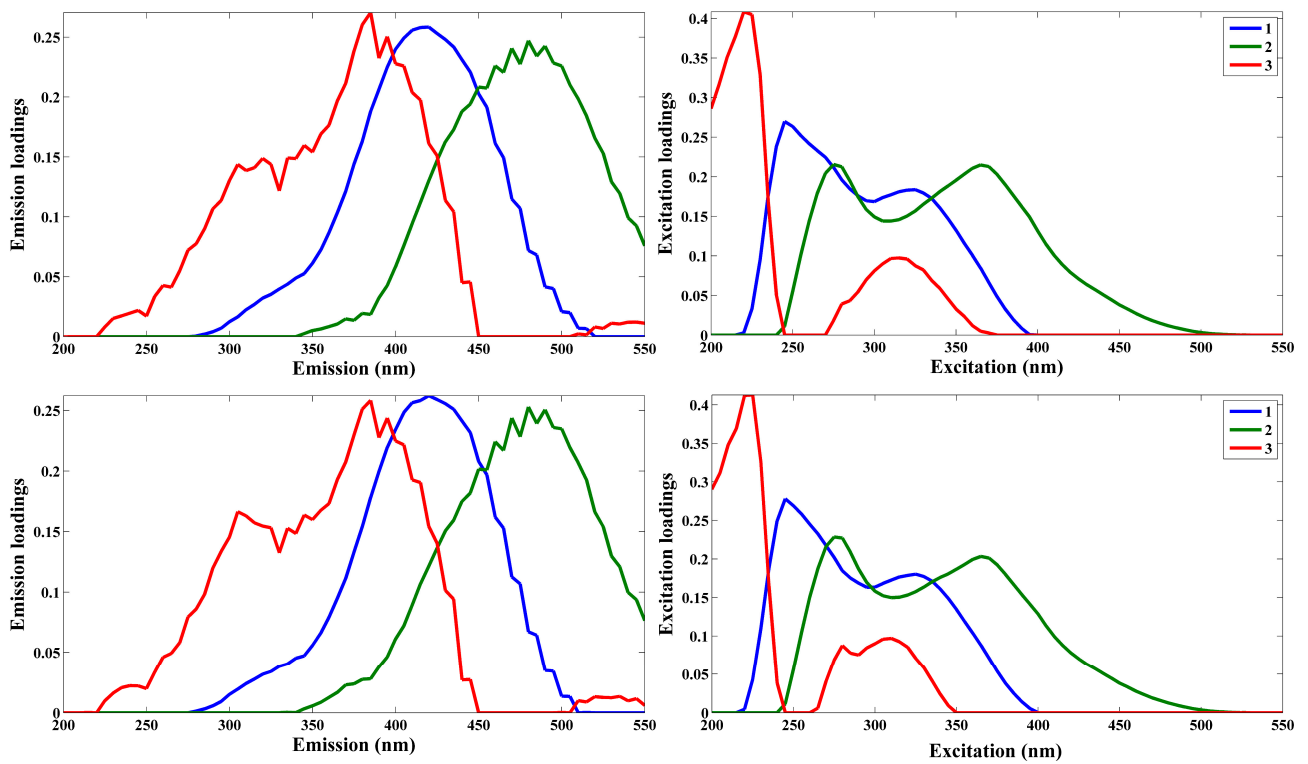


Figure 6. Split half validation of PARAFAC model A. When the fits of the splits are similar to each other and the entire model, a high stability of the model and low randomness of the fluorophores are given.

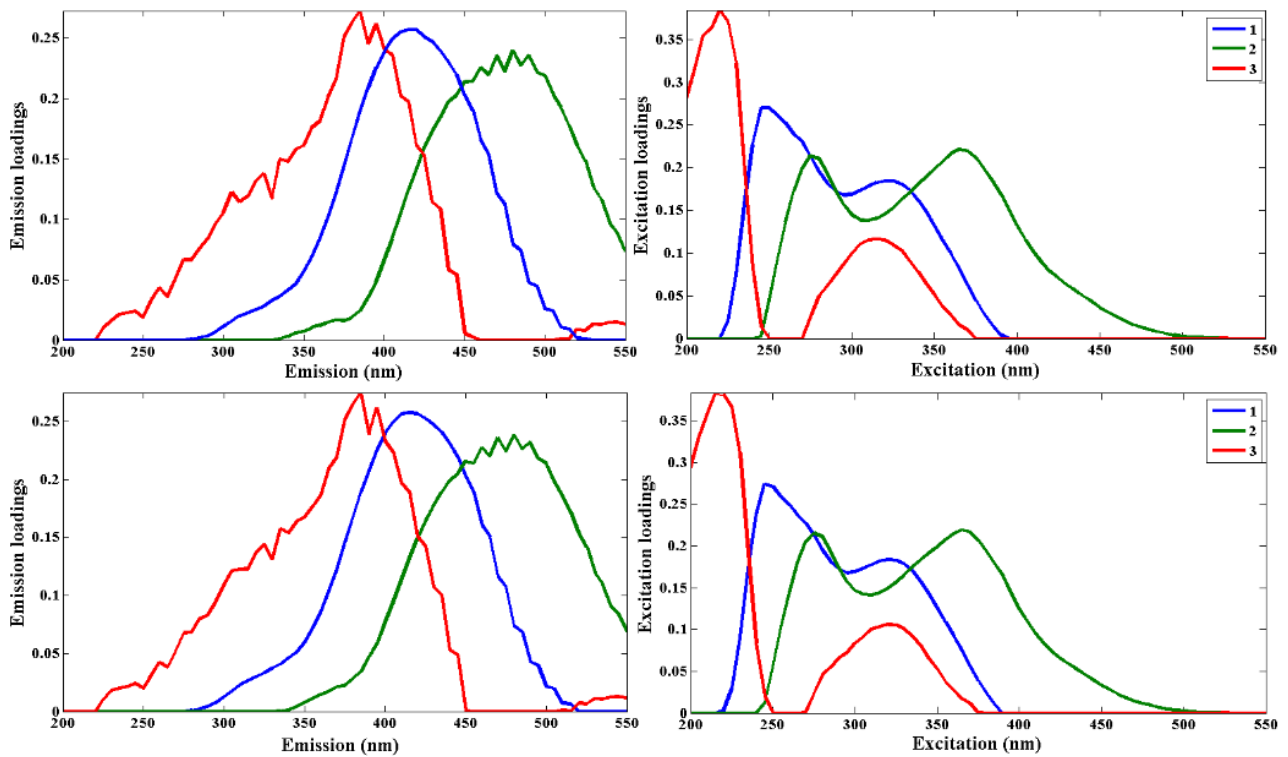


Figure 7. Split half validation of PARAFAC model B. When the fits of the splits are similar to each other and the entire model, a high stability of the model and low randomness of the fluorophores are given.

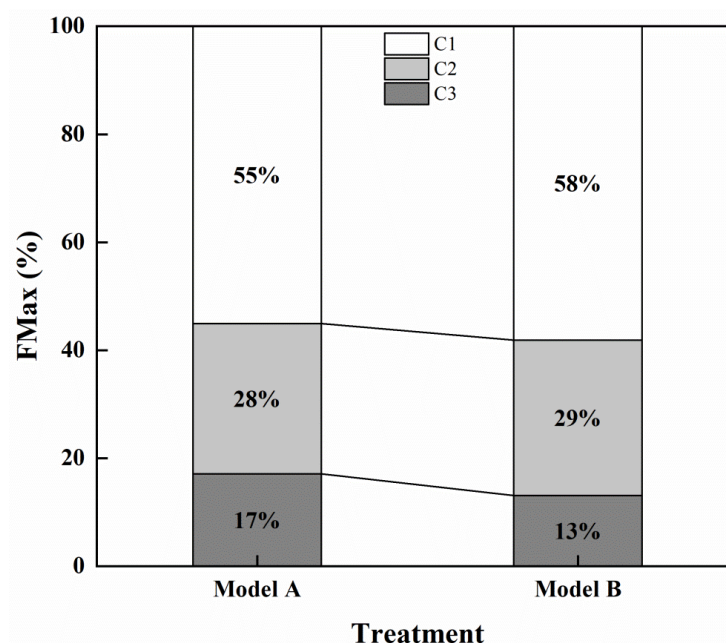


Figure 8. The percentage of PARAFAC-derived components in three surface soil DOM from temperate forest. Model A, the control group; Model B, the group with N addition. C1, fulvic-like constituent; C2, humic-like constituents; C3, protein-like constituent.

4. Conclusions

Deploying UV-vis spectroscopy and EEM-PARAFAC modelling to study DOM in soil reveals the influence of the long-term addition of N to forest soil on DOM in the form of changes in its properties and provides a reference for further ecosystem C storage research. The addition of N greatly increases the content of DOC in forest soils, and the magnitude of that increase depends on the form and the dose of N. The spectral characteristics of DOM revealed more detailed structural changes. Although the addition of N did not alter the fluorophore position in the DOM in forest soil, it did affect the proportions of the three PARAFAC-derived components in the DOM. The characterization parameters achieved through UV-vis and EEM spectroscopy showed that adding N makes DOM more aromatic and increases the degree of its humification. Our observation suggested that N addition made soil DOM a more complex microstructure and difficult to decompose and release, which might be beneficial for the maintenance of soil fertility and effective for soil organic carbon sequestration. Considering the amount ($30.6 \text{ kg N ha}^{-1} \text{ year}^{-1}$) of local actual N deposition and the decrease in $\text{NH}_4^+ \text{-N}/\text{NO}_3^- \text{-N}$ in the composition of N deposition, we inferred that there might be many other ecological consequences of DOM in our study area in the future, which should attract the widespread attention of scientists and the public.

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